SECM Imaging of Patterned Mammalian Cells

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Microbiochips containing arrays of immobilized oligonucleotides or proteins has been appeared to be the most promising technology for high-throughput analysis in medical biology. Our group has recently reported an antibody-chip combined with scanning electrochemical microscopy (SECM), [1] which is a kind of scanning probe microscope equipping a microelectrode to afford images of local electrochemical properties.

In the present work, we attempt to fabricate a microbiochip based on arrays of mammalian cells. We have already reported that the SECM imaging of the reduction current of oxygen around cells is one of suitable ways to clarify the influences of chemical stimulations on the cellular status. [2] In order to fabricate a bio-chip-device using living cells as a sensing element, however, it is need to place cells in predetermined locations with defined shapes and sizes. The "Micro-Contact-Printing" developed by Prof. Whitesides and his coworkers is the technique suiting for the fabrication of such cell-chip, [3] while most of their research are based on thiol-SAM on a gold substrate. Since such conductive substrate is often troublesome for SECM imaging, we modified their procedure to achieve direct stamping of proteins on an insulating glass substrate.

Polydimethylsiloxane (PDMS) stamp (Figure 1a) was prepared by using a photoresist pattern as a template, and pretreated with O_2 plasma to make its surface hydrophilic. A fibronectin (FN) solution (2.8×10^{-3} mg/ml) was dropped onto the stump surface (0.1 ml / cm^2) and dried for 5 min. The resulting FN-coated stamp was put on a hydrophobic glass plate and incubated for 1 hour. After rinsing throughly with PBS, the substrate was further incubated in BSA / PBS solution (0.3 mg / ml). The blocking effect of BSA layer to the cell adhesion was previously examined by quartz crystal microbalance (QCM). The resulting protein-patterned substrate was then incubated in the culture medium containing cell suspension of HeLa cells (3×10^5 cells / ml). Figure 1b shows microscope image of HeLa cells that have been selectively attached to the pattern of fibronectin. The SECM imaging was carried out in a 10 mM HEPES / 160 mM NaCl buffered solution, as described previously. A Pt-disk microelectrode (5 um in radius) was scanned over the cell pattern (10 um above the substrate) with monitoring oxygen at -0.5 V vs. Ag/AgCl. Figure 1c shows the SECM images of the patterned HeLa cells. The each area with low oxygen reduction current (dark areas) coincides roughly with the cell pattern, giving the respiratory activity of cells. We prepared also a line-and-space pattern of HeLa cells and observed higher respiratory activity, while the experiments were preliminary.

The patterning of bovine aorta endothelial cell (BAEC) has been also attempted for investigating the contact inhibition of cell proliferation and the nature of cell-cell junction within the endothelial tissue.

Acknowledgement: This study was supported by Industrial Technology Research Grant Program in '00 from NEDO, and partly by Grant-in-Aids for Scientific Research on Priority Area (No. 11227201) from the Ministry of Education, Science and Culture, Japan..

References:

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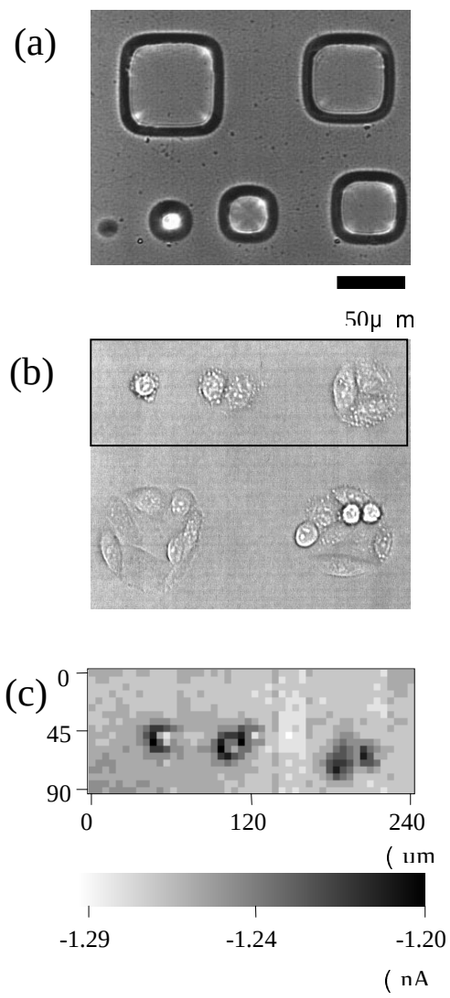

Figure 1
(a) A photograph of PDMS stamp used in this study. (b) A microscope image of the patterned HeLa cells on a glass plate. (c) SECM image based on the reduction current of oxygen, taken for the enclosed part of (b).